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SalPep Biotechnology Inc.
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CANADA

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If the applicant is a corporate body, give the country/state of its incorporation

CANADA

4. Title of the invention

Novel Peptides for Treatment of Inflammation and Shock

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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NOVEL PEPTIDES FOR TREATMENT OF INFLAMMATION AND SHOCK

Field of Invention

This invention relates to novel peptides which regulate cardiovascular and immunological responses to anaphylactic and endotoxic shock.

Background of Invention

Cardiovascular Shock and Immune System Dysfunction

Infections, trauma (e.g. falls, automobile accidents, gun and knife wounds), cardiovascular accidents (e.g. aneurysms and ischemic events often associated with surgery) and endogenous inflammatory reactions (e.g. pancreatitis and nephritis) often leads to profound dysfunction of the homeostatic mechanisms involved in regulating cardiovascular and immune system function. Aside from immediate cardiovascular collapse, caused by haemorrhaging or anaphylaxis, several conditions such as ischemia and infections through inappropriate and/or excessive activation of the immune system often results in cardiovascular dysfunction that develops over a period of hours to days. In all cases compromised cardiovascular function increases morbidity and is life threatening.

Sepsis and the Systemic Inflammatory Response Syndrome

Acute and chronic infections are a major challenge to current critical care medicine. Sepsis is diagnosed largely on observed physiological changes such as increase (fever) or decrease in body temperature (hypothermia), increased heart rate (tachycardia), increased respiration rate (tachypnea), elated or diminished white blood cell counts and inadequate perfusion of tissues and organs. Changes in blood pressure are not included in the definition because they occur late in the course of the syndrome. Decreases in blood pressure reflect the development of shock, and contribute to multiple organ failure, a leading cause of death in these patients. It is apparent that within this definition of sepsis a large number of patients are included who exhibit similar physiological signs but have no evidence of any type of infection (viral, fungal, parasitic, fungal or bacterial).

Other precipitating causes include: pancreatitis, burns, ischemia, multiple trauma and tissue injury (often due to surgeries and transplants), haemorrhagic shock and immune-mediated organ dysfunction. These conditions induce a sepsis-like condition.

A clinical useful paradigm has been developed that helps define patients that could possibly progress into sepsis or sepsis-like conditions, and eventually shock. This paradigm is called the systemic inflammatory response syndrome (SIRS).

SIRS is defined clinically as a patient who exhibits two or more of the following criteria:

1) a body temperature greater than 38°C or less than 36°C;
2) a heart rate greater than 90 beats/minute;
3) a respiratory rate greater than 20 breaths/minute;
4) a white blood cell count greater than 12 million/ml or less than 4 million/ml.

Some 68% of all patients entering the hospital possess SIRS, and thus are at risk for the development of sepsis or a sepsis-like condition and shock. A confirmed infectious process (i.e. positive blood cultures) are required for the rigorous diagnosis of sepsis. Nonetheless, some infectious negative patients progress to a stage of severe sepsis, which is defined by the presence by one of the following conditions:

1) a reduction of systolic blood pressure to less than 90 mm Hg;
2) a systemic manifestation of poor tissue perfusion as reflected by lactic acidosis, low urine output or acute alteration of mental state.

A SIRS patient can progress directly to severe sepsis, in the absence of a definable infectious agent. Some of the conditions that favour progression to the severe sepsis stage include: pancreatitis, burns, and cerebral or spinal injuries. A patient is considered to be in shock if he/she remains hypotensive (i.e. systolic blood pressure below 90 mm Hg) following the administration of 500 ml of fluid.

The progression from SIRS to sepsis to severe sepsis and eventually shock can occur if the SIRS is not arrested. The associated mortality rates for the four

different stages are: SIRS (7%), sepsis (16%), severe sepsis (20%) and shock (46%). Thus, it is readily apparent that the effective treatment of SIRS and the subsequent more severe inflammatory responses would most readily be treated by interventions at a very early stage in the disease process, ie. demonstration of SIRS.

Economical Impact of Sepsis and Systemic Inflammatory Response Syndrome

Sepsis syndrome is the 13th leading cause of death in the USA. Some 10% to 15% of patients will either arrive at the hospital or develop in the hospital a clinically important infection. These percentages translate into 500,000 patients per year in the USA of whom 50% are infected with Gram-negative bacteria. On the average 40% of these patients die within 28 days of admission to the Intensive Care Unit. The survivors often have extended periods of hospital care. These numbers indicate the high costs incurred by sepsis due to long-term use of expensive hospital facilities and mortality and disability payments by life insurance companies. Currently, in the USA 175,000 cases of sepsis a year with an average hospital stay of 14 days at a minimum of $1,200.00/day costs the US economy nearly $3 billion dollars. Drug costs are not included in this figure.

Current Therapeutic Strategies for the Treatment of Sepsis and Systemic Inflammatory Response Syndrome

As evidenced by the high mortality rates no effective therapy has been developed for the treatment of sepsis and SIRS. The standard therapies for sepsis involve administration of antibiotics to bring the infection under control and fluid/colloid therapy to maintain circulating blood volume. Frequently, drugs which help maintain blood pressure, such as dopamine and vasopressin, are also administered. With recent advances in our understanding of the haematological and immunological mechanisms associated with sepsis/SIRS, a variety of new strategies and therapies are being tested in laboratories and Intensive Care Units around the world. The search for an effective treatment of sepsis is a very active area of research involving many major pharmaceutical firms and biotechnology companies. Some agents that have been and are currently being evaluated clinically for the treatment of sepsis include: monoclonal antibodies against the

lipid core of lipopolysaccharide, monoclonal antibodies against tumour necrosis factor alpha (TNFα), receptor conjugates of TNFα, and antagonists against interleukin-1 (IL-1). None of these products are currently available outside clinical trials in North America, although a monoclonal antibody against LPS is available for clinical use in Europe. Serious problems have been encountered with some of these products, since the confirmation of initial claims of efficacy of treatment was not proven in larger studies.

Recent strategies for developing more targeted therapies for the treatment of sepsis have been disappointing. In addition, many molecules in the new generation of anti-septic agents are very expensive and most possibly produce untoward immunological and cardiovascular reactions which make them contra-indicated in some cases of non-bacteremic shock.

Anaphylaxis

Anaphylactic reactions are severe manifestations of an immunological protective mechanism, primarily designed to prevent entry and eliminate foreign molecules from the body. These so called hypersensitivity reactions to environmental antigens have a role in the etiology of asthma, rhinitis, urticaria (hives), eczema and certain gastrointestinal disorders. If sufficiently intense hypersensitivity reactions can result in a profound decrease in blood pressure. This shock can not only lead to immediate death, but also facilitate the development of multiple organ pathology and even failure (involving the lungs, liver, kidneys and intestine) that requires extensive medical intervention.

Current Therapeutic Strategies for the Treatment of Anaphylaxis

The most severe cases of hypersensitivity reactions result in anaphylactic shock and are treated by the administration of fluids and vasoactive agents in order to restore blood pressure. When the hypersensitivity reaction is more localised, as in the case of asthma, prophylactic treatment with anti-inflammatory steroids, and symptom treatment with smooth muscle relaxing agents (e.g. β-adrenergic drugs) are particularly effective. Nonetheless, the most effective prophylactic treatment of hypersensitivity reactions is avoidance of the precipitating agent or antigen.

There remains a need for inexpensive and effective agents for treatment of cardiovascular shock, sepsis, systemic inflammatory response syndrome and anaphylaxis.

Brief Description of the Drawings

Figure 1 shows the effect of SGP-T on the average decrease in mean arterial blood pressure (MABP) over a 1h period induced by intravenous LPS (*Salmonella typhosa*, 3.5 mg/kg), evaluated in pentobarbital anesthetized unoperated (■) and sialadenectomized (▲) rats. The rats were pretreated with SGP-T 1h prior to injecting the LPS. The reduction of the endotoxic hypotension was maximal at 1 μg/kg of peptide with sialadenectomized rats, but a larger dose (100 μg/kg) was required with unoperated rats. *$p < 0.05$

Figure 2 shows average decrease in MABP in rats (sensitised 1 month previously with 3000 larvae of the nematode *Nippostrongylus brasiliensis*) challenged by injection of worm antigen (100 worm equivalents), after the following treatments:

A: no drug

B: 10 μg SGP-T

C: 35 μg SGP-T

D: 100 μg SGP-T. *$p < 0.05$

Figure 3 shows hooded-Listar rats sensitized 3 weeks previously with egg albumin (EA) and equipped 1 week prior to experimentation with electrodes on their jejunum to record migrating myoelectric complexes (MMC's). The rats were either treated with 0.9% saline or one of three doses of SGP-T 30 minutes prior to a challenge with EA directly into the duodenum. All rats pretreated with saline and then challenged with EA exhibited disruption of their MMC's (solid bars) and 90% of them developed diarrhea (hatched bars) shortly after challenge. Sensitized rats challenged with bovine serum albumin (BSA), a naive antigen, did not exhibit anaphylactic symptoms. Rats pretreated with SGP-T at 10 and 100 μg/kg prior to challenge with the sensitizing antigen EA exhibited significant protection against intestinal anaphylaxis. Significance: **greater than 10 and 100 μg/kg SGP-T; *greater than 100 μg/kg SGP-T.

Figure 4: intravenous SGP-T at doses of 35 and 100 μg/kg reduced significantly the number of neutrophils migrating into a carrageenin-soaked sponge placed subcutaneously in the intra-scapular region of Sprague-Dawley rats 24 h previously.

Figure 5: neutrophils were squeezed out of sponges placed subcutaneously in the intra-scapular region of Sprague-Dawley rats and tested for superoxide production (O_2^-) in response to phorbol myristate acetate (PMA). Neutrophils extracted from sponges soaked only in 0.9% saline (sal) generated 14-15 nmoles of O_2^-/min/10^7 cells. The O_2^- production by neutrophils obtained from carrageenin-soaked sponges (carr) was significantly lower; ~4 O_2^-/min/10^7 cells. Treatment of the rats with intravenous SGP-T at the time that the carrageenin-soaked sponges were implanted dose-dependently reversed the inhibitory effect of carrageenin on the ability of PMA to stimulate O_2^- production.

Figure 6 shows superoxide anion production by human (■) and rat (▲) neutrophils treated with SGP-T.

Description of the Invention

The following abbreviations are used in the following description of the invention:

IFN:	interferon
IL:	interleukin
LBP:	lipopolysaccharide binding protein
LPS:	lipopolysaccharide
SIRS:	systemic inflammatory response syndrome
SGP-S:	submandibular gland peptide-S
SGP-T:	submandibular gland peptide-T
TNFα:	tumour necrosis factor alpha

Submandibular Gland Peptides

The salivary glands are classically viewed as accessory digestive glands which mediate their actions through exocrine secretion, although appreciation has grown recently for the importance of their endocrine secretions (1-3). The exocrine excretion for biologically active peptides from the salivary glands are essential for the health of the mouth (4), and whereas their endocrine secretions

help maintain the structure and function of a large variety of internal tissues and organs such as the digestive tract (5-7), the mammary glands (8), the liver (9-11), and the reproductive tract (12, 13). Another important action of salivary endocrine secretions is the modulation of the immune system, with effects being observed on lymphocyte (14,15), mast cell (16) and neutrophil (17,18) functions. The submandibular glands also regulate inflammatory reactions associated with the late-phase pulmonary inflammation induced by allergen in sensitized rats (19-21), and their removal exacerbates the severity of the hypotensive responses induced by intravenously administered lipopolysaccharide (LPS) (22).

The inventor has isolated from submandibular glands, and characterised, two novel peptides which have profound effects on a variety of cardiovascular and immunological perturbations and provide new therapeutic agents for treatment of a variety of disorders.

For example, SGP-T produces

(a) an approximately 50% reduction of lipopolysaccharide-induced hypotension, at doses as low as 1 μg peptide/kg body weight;

(b) a reduction of anaphylactic hypotension in egg albumin-sensitised animals, and also in nematodes;

(c) a protective effect against ventricular dysfunction during systemic anaphylaxis;

(d) attentuation of antigen-induced pertrubations of gastrointestinal motility in ovalbumin-sensitised animals;

(e) a significant reduction of *in vitro* antigen-induced smooth muscle contraction, in muscle from ovalbumin-sensitised animals;

(f) a significant reduction of the fever provoked by bacterial endotoxin;

(g) an approximately 50% inhibition of neutrophil migration;

(h) a prevention of carrageenin-induced inhibition of superoxide production by phorbal myristate (PMA) and *f*MLP; and

(i) non-competitive blocking of the effect of the protein kinase inhibitor, staurosporine.

In accordance with one embodiment, the invention provides novel peptides of the formula H_2N-X_1-X_2-X_3-X_4-X_5-A-COOH wherein

X_1 is an aliphatic amino acid residue having a side chain hydroxyl group,

X_4 is an aromatic amino acid residue,

X_5 is an acidic amino acid residue,

A is a sequence of 1 to 3 amino acid residues which are the same or different and are aliphatic amino acid residues,

and X_2 and X_3 are the same or different and are any amino acid residue.

These peptides are effective to prevent, reduce or reverse the hypotension associated with an anaphylactic and endotoxic shock, prevent cardiovascular and intestinal anaphylaxis and inhibit neutrophil migration.

In accordance with a further embodiment, A is a sequence of 1 to 3 amino acid residues which are the same or different selected from the group consisting of glycine, proline, sarcosine, azetidine, nipecotic acid and pipecotic acid.

In accordance with a preferred embodiment, the invention provides a novel peptide designated submandibular gland peptide-T or SGP-T, which has the amino acid sequence

NH_2-Thr-Asp-Ile-Phe-Glu-Gly-Gly-COOH (TDIFEGG).

SGP-T reverses the hypotension associated with anaphylactic and endotoxic shock, prevents cardiovascular and intestinal anaphylaxis and inhibits neutrophil migration.

In accordance with a further embodiment, the invention provides a novel peptide designated submandibular gland peptide-S or SGP-S, which has the amino acid sequence

NH_2-Ser-Gly-Glu-Gly-Val-Arg-COOH (SGEGVR).

SGP-S reverses the hypotension associated with endotoxic shock. Neither peptide affects blood pressure in non-shocked animals.

In accordance with a further embodiment, the invention provides effective fragments and analogues of peptides SGP-T and SGP-S.

The invention provides novel peptides of the formula

H_2N-X_1-X_2-A-COOH wherein

X_1 is an aromatic amino acid residue,

X_2 is an acidic amino acid residue, and

A is a sequence of 1 to 3 amino acid residues which are the same or different and are aliphatic amino acid residues.

In accordance with a further embodiment, the invention provides peptides having the amino acid sequence

(a) Phe-Glu-Gly; and

(b) Phe-Glu-Gly-Gly-Gly.

Peptides SGP-S and SGP-T are synthesised in submandibular glands and correspond to previously unidentified portions of a submandibular protein described by Rosinski-Chupin et al. (*DNA Cell Biol.*, v. 9, pp. 553-559 (1990); P.N.A.S. USA, v. 85, pp. 8553-8557 (1988)).

The invention enables pharmaceutical compositions comprising peptide SGP-T or peptide SGP-S or an effective fragment or analogue of one of these peptides for prevention and/or treatment of inflammation, cardiovascular shock including anaphylactic shock and endotoxic shock and systemic inflammatory response syndrome (SIRS).

The invention also enables methods of preventing and/or treating inflammation, cardiovascular shock including anaphylactic shock and endotoxic shock and systemic inflammatory response syndrome (SIRS) by administration of an effective amount of peptide SGP-T or peptide SGP-S or an effective fragment or analogue of one of these peptides to a mammal in need of such prevention and/or treatment.

Peptides SGP-T and SGP-S and effective fragments or analogues thereof may be administered prophylactically to treat SIRS patients before they progress into shock or may be administered after shock has developed, to combat hypotension.

Peptides SGP-T and SGP-S and effective fragments or analogues thereof may also be used to arrest the progression of mild anaphylaxis to more severe systemic inflammation.

Peptide SGP-T and effective fragments and analogues thereof may be used to prevent the development of anaphylaxis in susceptible subjects, for example, individuals who experience anaphylactic reactions to insect bites or in food allergies.

Peptide SGP-T and effective fragments and analogues thereof may be used to modulate neutrophil function, in a variety of inflammatory events involving neutrophil infiltration, such as post surgical ischaemia and pancreatitis.

The peptides of the invention, and fragments and analogues thereof, may be synthesised conveniently and cheaply by conventional peptide synthetic techniques. Furthermore, the peptides of the invention, by virtue of their small size, are likely to produce less immunological reaction in treated subjects than therapeutics such as monoclonal antibodies.

The invention also includes effective pseudo-peptides or peptide mimetics derived from the peptides of the invention.

EXAMPLES

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of molecular genetics, protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

Example 1

Isolation, Purification and Sequencing of SGP-T and SGP-S

The purification procedures involved homogenization of the glands in 0.1N HCl, centrifugation of the extract at 15,000g for 1h, sequential molecular weight cut-off filtration of the supernatant with Amicon 30,000, Centricon 10,000 and 3,000 filters followed by 5 steps of reverse phase high performance liquid chromatography (RP-HPLC) using 20 to 50% acetonitrile. At each step of the purification biologically active fractions were identified by monitoring their ability to reduce LPS-induced hypotension in sialadenectomized rats. The two peptides were sequenced at the Protein/Peptide Synthesis Unit of The University of Calgary and the Alberta Peptide Institute at The University of Alberta,

Edmonton, Alberta. The peptides were then synthesized, Sephadex G-10 purified and their amino acid composition confirmed.

SGP-T and SGP-S

Peptides SGP-S and SGP-T have the following amino acid sequences:

SGP-S: NH_2-Ser-Gly-Glu-Gly-Val-Arg-COOH (SGEGVR);

SGP-T: NH_2-Thr-Asp-Ile-Phe-Glu-Gly-Gly-COOH (TDIFEGG)

Example 2

Effects of SGP-T and SGP-S on Experimental Endotoxemia

The animal model of endotoxic shock used involves intravenous injection of endotoxin (3.5 mg/kg of LPS from *Salmonella typhosa*) into pentobarbital anaesthetized Sprague-Dawley rats. The endotoxin was injected slowly over 1 min via the jugular vein, and MABP was monitored continuously for 60 min, with the average blood pressure at 15, 30, 45 and 60 min being calculated. Studies were performed on unoperated rats and rats with their submandibular glands removed (sialadenectomized).

The results shown in Table 1 are the averages of three different experiments using the protocol defined above. SGP-S (100μg/kg) and SGP-T (100μg/kg) were administered either 90 min before LPS, 30 min before LPS or 10 after LPS. The sialadenectomized rats exhibited a more severe hypotensive response to LPS than unoperated rats. The average MABP for the 60 min following LPS injection (MABPaft) for the sialadenectomized rats (68.03±3.4 mm Hg) was significantly less than that seen with unoperated rats (88.03±3.6 mm Hg). Neither SGP-T nor SGP-S, when given prior to the LPS challenge, had appreciable effects on MABP.

With unoperated rats SGP-T effectively prevented the drop in MABP elicited by LPS, and this effect was independent of time of administration of the peptide. Overall SGP-T reduced by 60% the decrease and the percent decrease in MABP, relative to pre-LPS values, induced by endotoxin. SGP-S, on the other hand, had no effect on the shock induced by endotoxin in unoperated rats.

With the sialadenectomized rats SGP-S, but not SGP-T, improved significantly the MABP after LPS, the decrease in MABP and the percent decrease in blood pressure due to endotoxin.

TABLE 1

Unoperated Rats

Treatment	MABPbef#	MABPaft	decMABP	%Dec
Saline (18)	122.3±3.9	88.0±3.6	-34.2±3.8	-27.6±2.5
SGP-T (18)	113.2±3.8	99.6±3.8*	-13.6±2.9**	-11.6±2.4**
SGP-S (15)	123.4±3.0	95.2±4.3	-28.1±1.8*	-22.9±1.3

Sialadenectomized Rats

Treatment	MABPbef	MABPaft	decMABP	%Dec
Saline (22)	125.0±3.8	68.0±3.4	-57.3±4.6	-44.7±3.1
SGP-T (18)	123.3±4.9	77.2±3.9	-46.1±4.9	-36.3±3.3
SGP-S (20)	119.7±1.8	81.6±5.8*	-37.6±4.4*	-32.3±3.9*

MABPbef = mean arterial blood pressure before LPS; MABPaft = MABP after LPS injection; dec MABP = decrease in MABP after LPS relative to MABPbef; %Dec = percent decrease in MABP relative to MABPbef. *different from Saline; ** different from Saline and SGP-S or SGP-T.

Example 3

Dose-Response Relationships and Endotoxin Induced Hypotension

In another series of experiments the dose response relationship for SGP-T and SGP-S in preventing endotoxin induced hypotension was examined. Figure 1 shows that SGP-T given intravenously 1.5 hours before LPS dose-dependently inhibited the decrease in blood pressure induced by the LPS in sialadenectomized rats. Whereas animals that were treated only with saline (no SGP-T) exhibited an average drop in blood pressure (MABP) of 55 mm Hg (millimetres of mercury) SGP-T at doses of 1, 3.5 and 10 μg/kg significantly prevented the LPS provoked drop in blood pressure. Doses of SGP-T outside this range were less effective in preventing the shock. The optimal dose for SGP-S in rectifying LPS-induced hypotension in unoperated rats was 100 μg/kg.

Example 4

Effects of SGP-T and SGP-S on Anaphylaxis

Cardiovascular Anaphylaxis

The effects of SGP-T on the anaphylactic hypotension were examined in Sprague-Dawley rats sensitized 5 weeks previously with the nematode parasite *Nippostrongylys brasiliensis*. Under pentobarbital anaesthesia worm antigen (100 worm equivalents) was injected and blood pressure followed for 1 hour. Whereas rats treated with the saline vehicle (no SGP-T) experienced a drop in blood pressure after antigen challenge of approximately 30 mm Hg (Figure 2), SGP-T, given 10 minutes prior to induction of anaphylaxis, dose-dependently protected against the anaphylactic hypotension.

Intestinal Anaphylaxis

SGP-T was also effective in preventing intestinal anaphylaxis *in vivo*. Figure 3 shows that in rats sensitized to egg albumin, instillation of the antigen (egg albumin) into the jejunum promoted diarrhea and disruption of normal fasting gut motility (migrating myoelectric complexes; MMCs). SGP-T, given intravenously at doses as low as 10 μg/kg attenuated these anaphylactic reactions and the larger dose of 100 μg/kg totally prevented the manifestation of anaphylaxis.

A similar protection against intestinal anaphylaxis was observed *in vitro* using isolated intestinal (the jejunum) segments obtained from egg albumin sensitized rats. SGP-T, at doses as low as 6.8×10^{-7}M reduced by 60% antigen induced contractions of these isolated intestinal tissues. This preparation was used to investigate the structure-activity relationships of SGP-T.

Example 5

Modulation of Neutrophil Function by SGP-T

The subcutaneous implantation of a carrageenin soaked sponge in rats is a model used to evaluate agents that modulate neutrophil chemotaxis, as carrageenin is a potent chemotactic agent and the sponge serves as a collecting reservoir. Intravenous injections of SGP-T inhibited neutrophil influx into the sponge in a dose-dependent manner (Figure 4). When the ability of neutrophils obtained from the carrageenin soaked sponges to generate superoxide anion was

evaluated those obtained from saline treated rats were totally refractory to *f*MLP and phorbol myristate acetate (PMA). On the other hand neutrophils collected from rats that had previously been treated with SGP-T were able to generate substantial amounts of superoxide (Figure 5).

Although the reasons for the lack of a superoxide response in carrageenin exposed neutrophils are unknown, receptor desensitization or uncoupling of the NADPH complex that generates the superoxide are possible explanations. SGP-T abrogates this desensitisation phenomena. By attenuating neutrophil chemotaxis and by conserving the oxidative capacity of neutrophils SGP-T may be an effective anti-inflammatory agent. Treatment with SGP-T would limit an excessive movement of neutrophils into an inflammatory site, prevent an excessive and intensive generation of superoxide, but still allow the neutrophils to exert oxidative capacity essential for their fight against inflammatory stimuli.

Example 6

Structure Activity Studies with SGP-T

By using rat jejunal segments obtained from egg albumin sensitized rats the analogues of SGP-T were tested for their ability to inhibit antigen (egg albumin) induced contractions of these tissues in isolated organ baths. Table 2 summarizes the amino acids that contribute to biological activity of SGP-T.

The parent molecule, SGP-T (T) had the most pronounced inhibition, and a comparable inhibition was obtained on replacing the Asp (D) with Ala (A). When Ile (I) was replaced with Ala (A) the analogue still reduced antigen-induced reductions by ~20%. Thus, neither Asp nor Ile are absolutely necessary for biological activity. Most other substitutions were found to be essential. An N-terminal Thr (T) is required since replacing this amino acid with Ala or placing a Ser (S) before the Thr totally abolished inhibitory activity. The Phe (F) and the Glu (E) are essential, as are a carboxy-C terminal. Glycines (G) are also essential for biological activity.

Table 2. Structure activity studies with SGP-T in inhibiting antigen induced contractions of egg albumin sensitized jejunal segments.

SGP-T: NH_2-Thr-Asp-Ile-Phe-Glu-Gly-Gly-COOH

		T D I F E G G	
5	T2:	STDIFEGG	-
	SGP-T:	TDIFEGG	+++
	T3:	ADIFEGG	-
	T7:	TAIFEGG	+++
	T10:	TDAFEGG	+
10	T11:	TDIAEGG	-
	T9:	TDIFAGG	-
	T5:	TDIFEGG-NH2	-
	T4:	TDIFE	-
	T6:	FEGGG	++
15	T8:	FEG	++

The signs indicate relative biological activity: +++ = highest inhibition (−60%), ++ = moderate inhibition (−40%), + = lowest inhibition (−20%), - = no inhibition.

The parent molecule, SGP-T (T) had the most pronounced inhibition, and
20 a comparable inhibition was obtained on replacing the Asp (D) with Ala (A).

Since some small peptides of the sequence FEGGG and FEG appeared in the amino acid sequencing these peptides were also tested for inhibitory activity against antigen induced contraction of sensitized jejunal segments. Both FEGGG and FEG inhibited the response by −40%. Thus, the sequence FEG is the
25 minimal requirement for inhibitory activity, and the addition of the Thr-Asp-Ile (TDI) sequence to FEG enhances the inhibition with the N-terminal Thr (T) being the critical amino acid.

Example 7

Acute effect of SGP-T on superoxide production of neutrophils

Neutrophils obtained from carrageenin soaked sponges implanted subcutaneously in the rats or from the blood of healthy human volunteers were preincubated with various doses of SGP-T for 30 minutes and then stimulated with 10^{-7}M PMA and the rate of superoxide anion production determined. At doses less than 1 μM, SGP-T inhibited superoxide anion production by both rat and human neutrophils, although the rat neutrophils were approximately 10-fold less sensitive than human neutrophils to this inhibitor effect (Figure 6). At higher doses of the peptide (>1 μM), an enhancement of superoxide anion production was evident with human neutrophils.

At 0.001 and 0.01 μM SGP-T, O_2^- production was inhibited by 15 to 20%, and 10-fold higher concentrations were required for such inhibition with rat neutrophils. Much higher concentrations of SGP-T (10 and 20 μM) stimulated O_2^- production by human neutrophils. Each value is presented as the mean ± SEM, and the number of experiments was between 6 and 12.

The present invention is not limited to the features of the embodiments described herein, but includes all variations and modifications within the scope of the claims.

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FIGURE 1

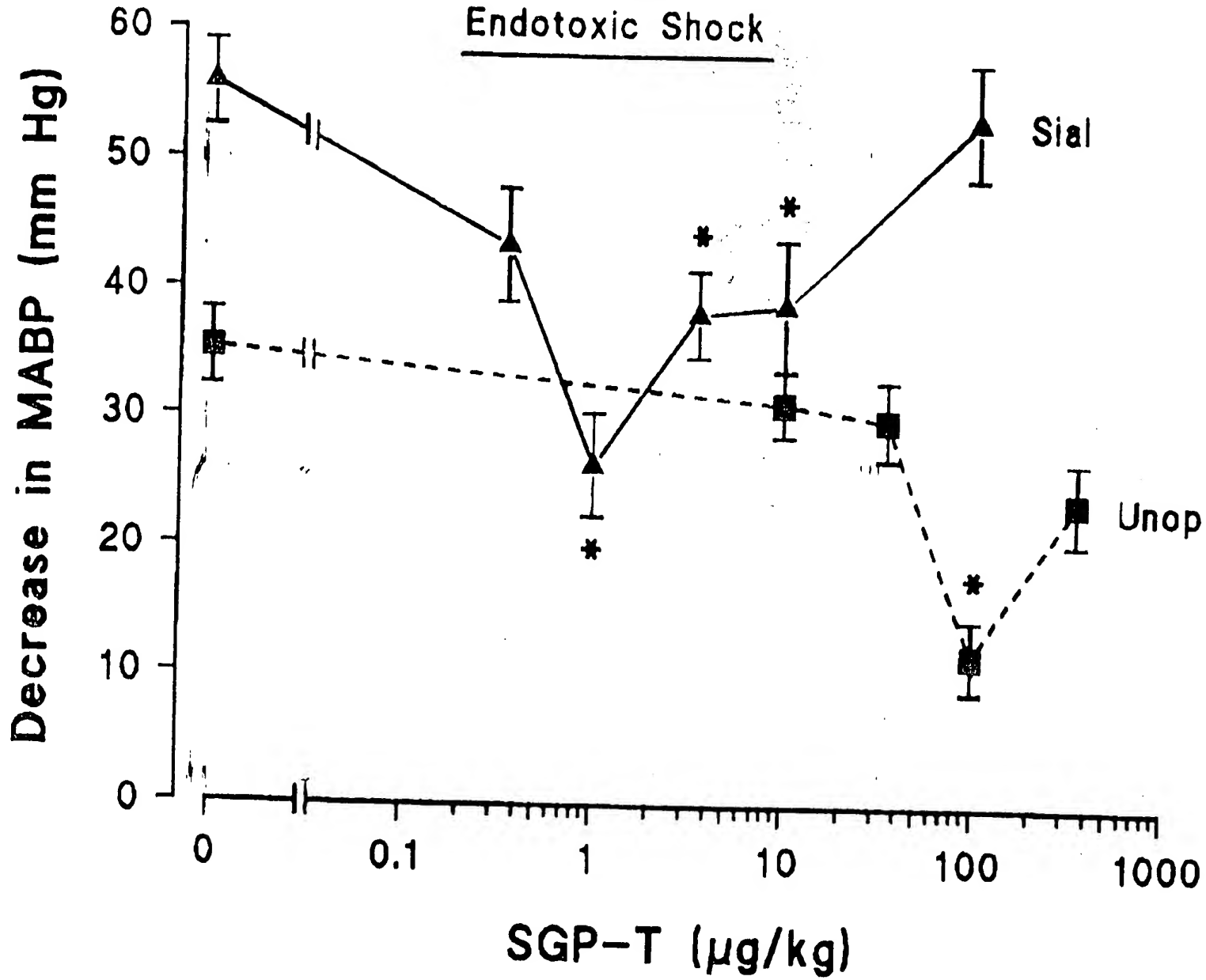

Endotoxic Shock
Decrease in MABP (mm Hg)
60
50
40
30
20
10
0
Sial
Unop
0
0.1
1
10
100
1000
SGP-T (µg/kg)

FIGURE 2

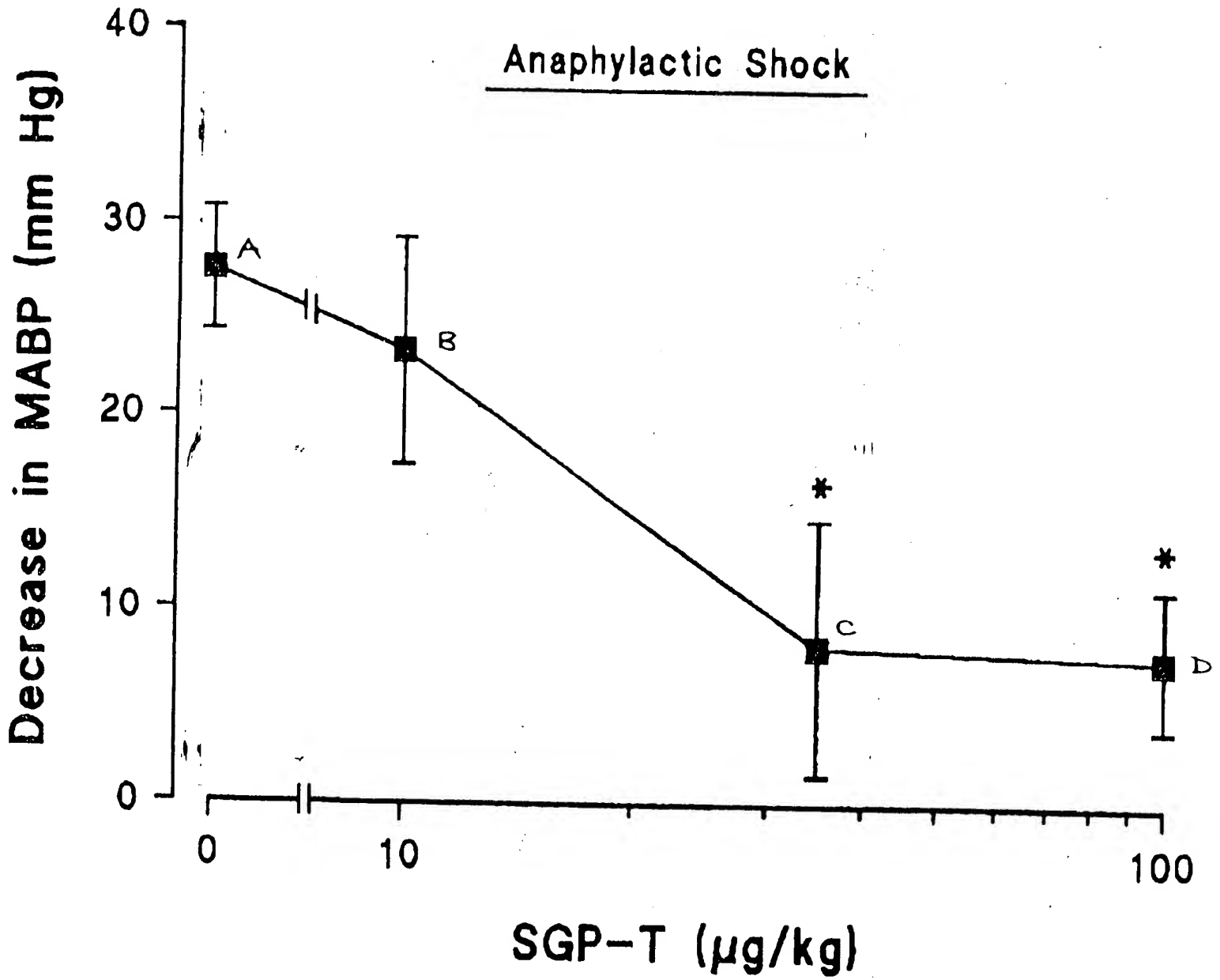

Anaphylactic Shock
Decrease in MABP (mm Hg)
40
30
20
10
0
A
B
C
D
*
*
0
10
100
SGP-T (µg/kg)

FIGURE 3

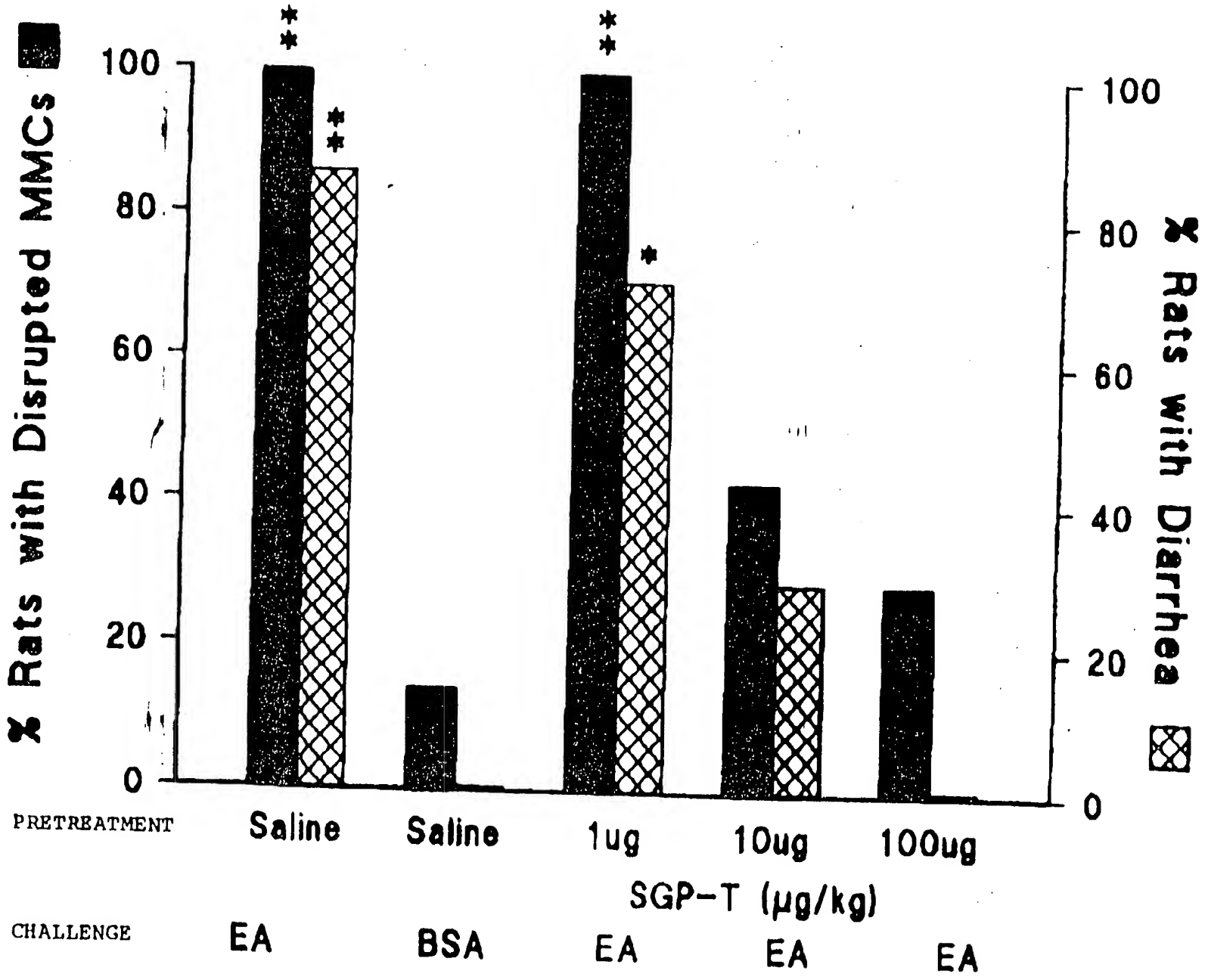

% Rats with Disrupted MMCs
100
80
60
40
20
0
% Rats with Diarrhea
PRETREATMENT
Saline
Saline
1ug
10ug
100ug
SGP-T (µg/kg)
CHALLENGE
EA
BSA
EA
EA
EA

FIGURE 4

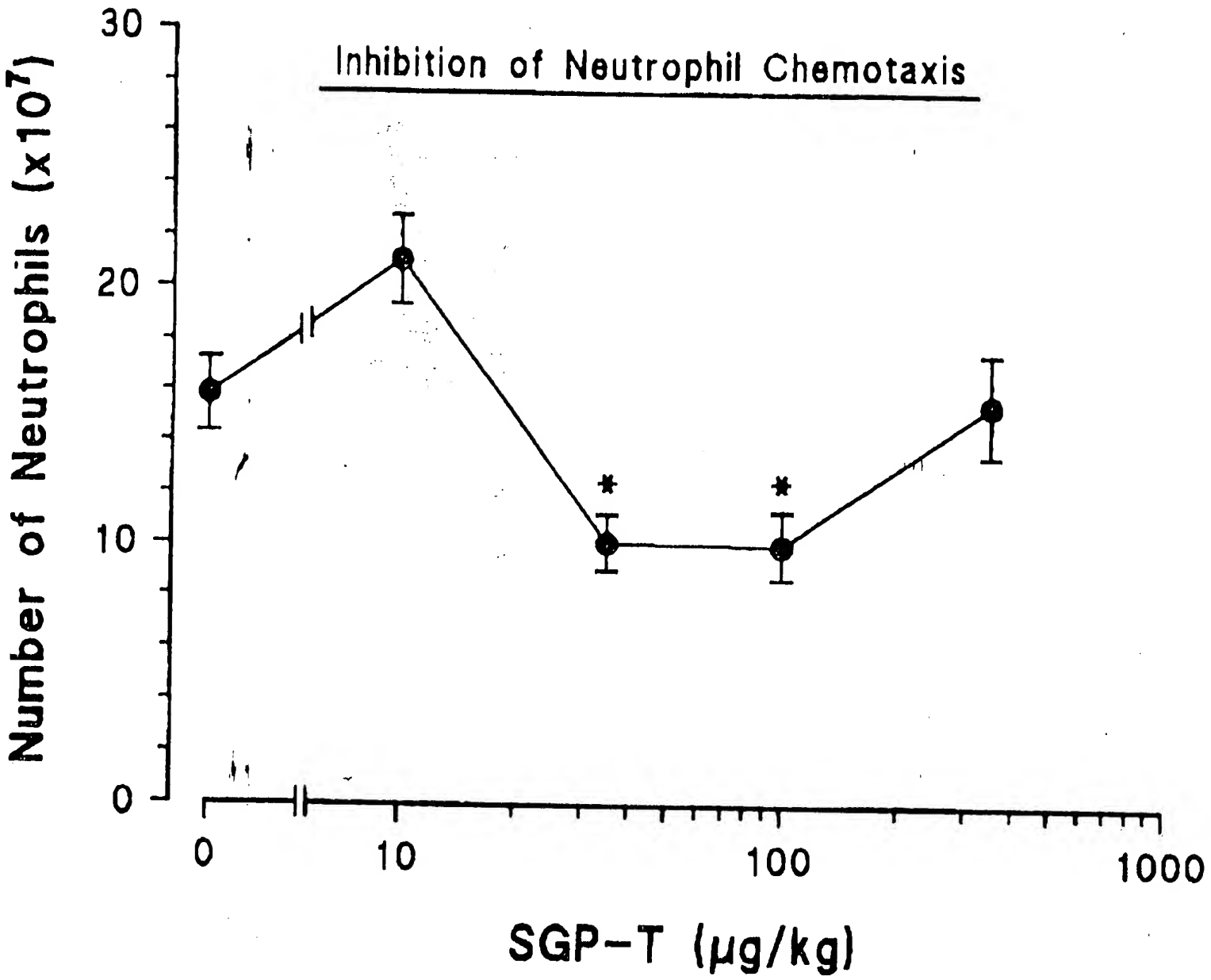

Inhibition of Neutrophil Chemotaxis
Number of Neutrophils (x10⁷)
30
20
10
0
*
*
0
10
100
1000
SGP-T (µg/kg)

FIGURE 5

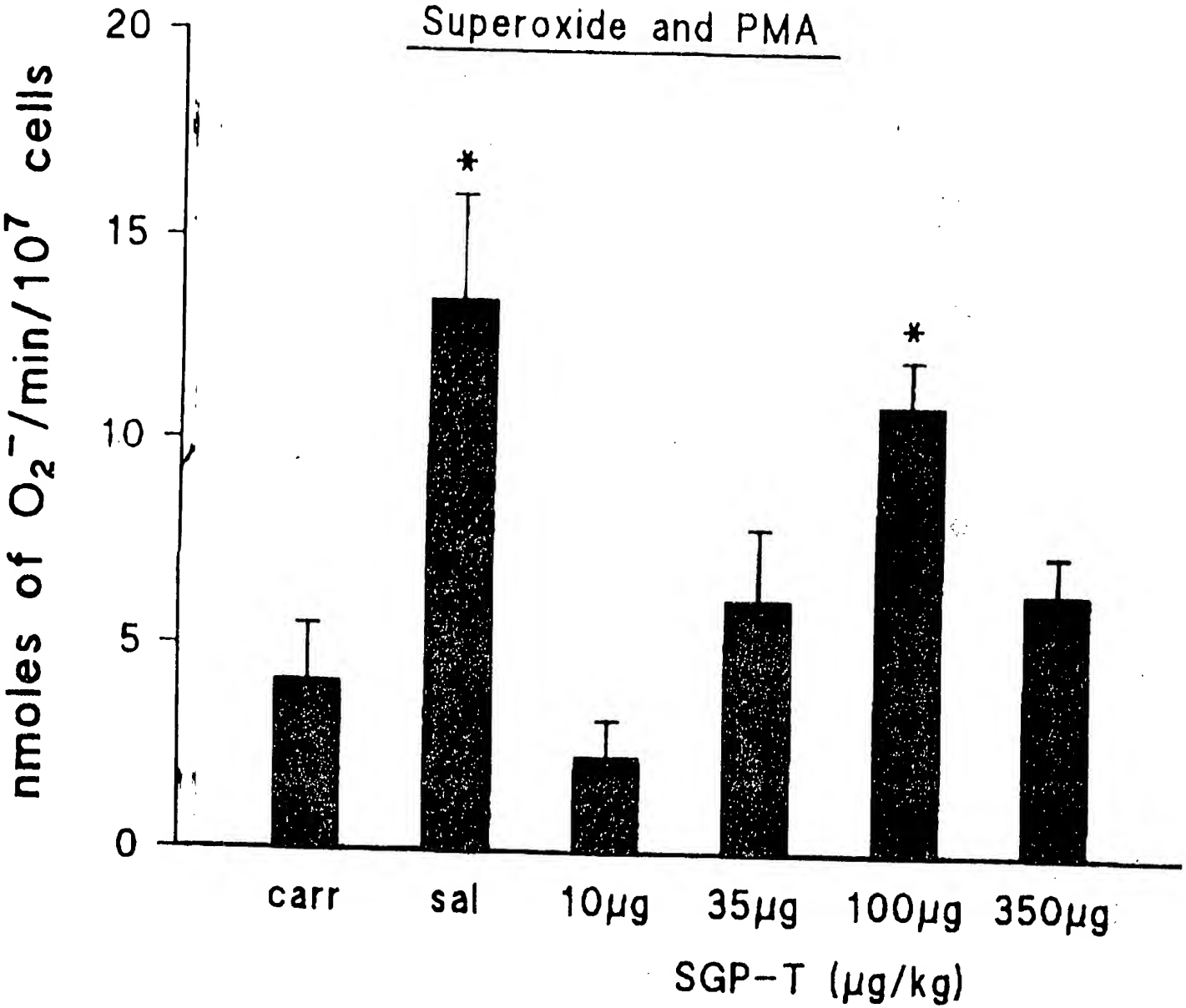
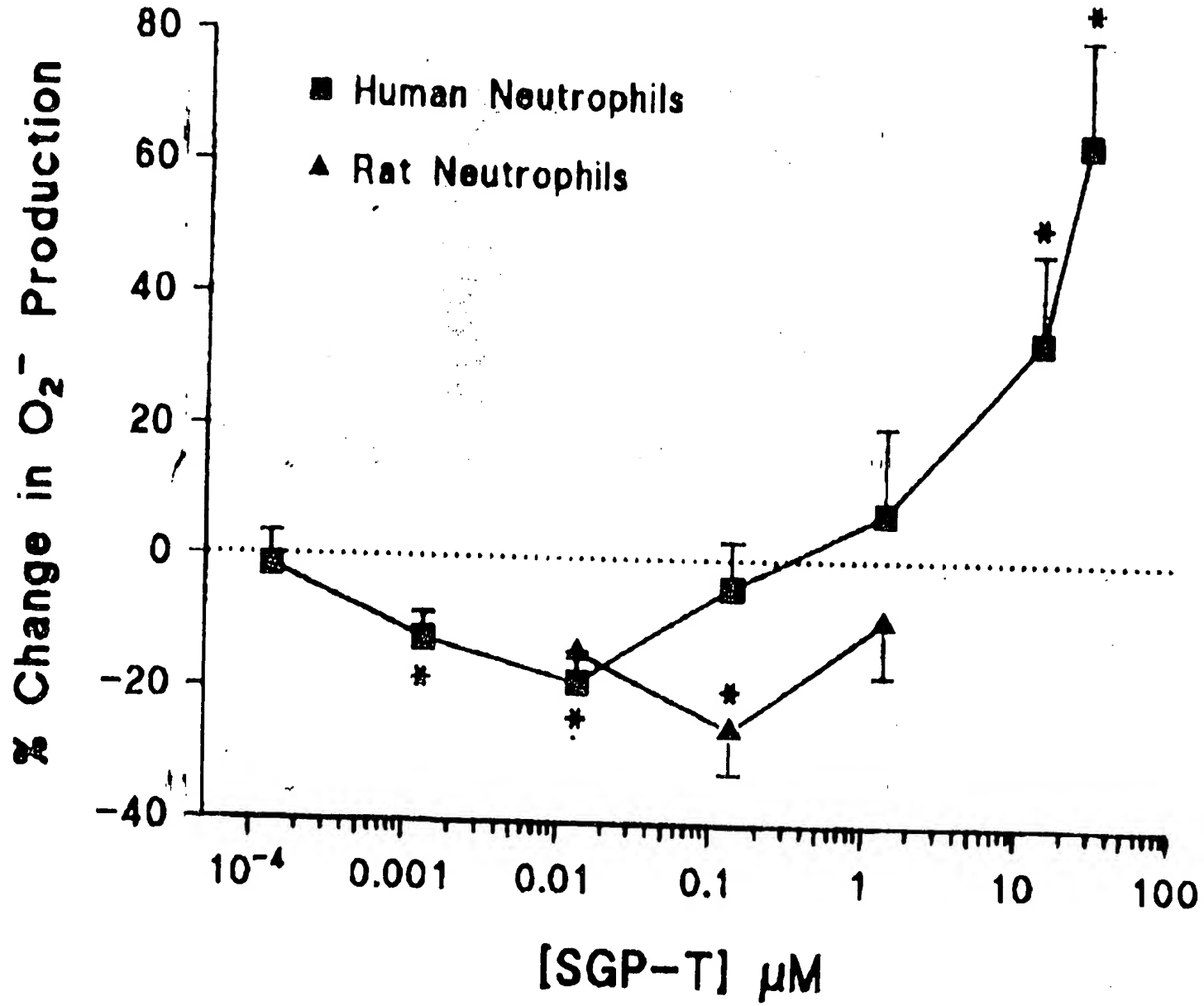

Superoxide and PMA
nmoles of O_2^-/min/10^7 cells
20
15
10
5
0
*
*
carr
sal
10µg
35µg
100µg
350µg
SGP-T (µg/kg)

FIGURE 6


80
60
40
20
0
-20
-40
% Change in O_2^- Production
Human Neutrophils
Rat Neutrophils
10^{-4}
0.001
0.01
0.1
1
10
100
[SGP-T] μM